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Country:	Denmark
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Activation of Lipid-Prot in Lung Surfactant Formulations

Field of the Invention

The present invention relates to the use of the surprising finding that a dynamic process of spreading takes place during a defined span of time following the reconstitution of a lyophilised lung surfactant in a physiological electrolyte solution. Hereby, a more active spreading of said surfactant into the alveoles is obtained, which in its turn opens the possibility to use this process also as a carrier for other therapeutic components into areas that are hard to access and thus provides an improved transport of compounds over the water-air surface of the lungs. The invention thereby offers a composition and a method for an improved treatment of respiratory distress syndrome (RDS) and other pulmonary diseases that are associated with deficiency of surfactant, as well as a more efficient means for the delivery of a number of therapeutic compounds for other diseases, as well.

Background

Pulmonary lung surfactant (LS) is a complex and highly surface-active material composed of lipids and proteins that is found in the fluid lining the alveolar surface of the lungs. Its principal property is to reduce the surface tension in the lungs, which is achieved through the presence of the lipids as an organized structure at the air-liquid interface in the alveoli. LS prevents alveolar collapse at low lung volume and preserves bronchiolar potency during normal and forced respiration (biophysical functions). In addition, it is involved in the protection of the lungs from injuries and infections caused by inhaled particles and microorganisms (immunological, non-biophysical functions). LS is synthesised and secreted by alveolar type II cells. (For a review, see Robertson and Taeusch, 1995.)

The constitution of a pulmonary lung surfactant may vary with various factors such as species, age, and general health conditions of the subject. Various natural and synthetic constituents can substitute for each other in a surfactant. Therefore, even a nonrigorous definition of what surfactant is and what should be included in a therapeutic surfactant is dependent on the situation. Surfactant isolated from lung lavage of healthy mammals contains about 10% protein (half of which is surfactant specific), and 90% lipids, of which 80% is phospholipid and 20% is neutral lipid, including 10% unesterified cholesterol. The

phospholipid fraction contains mostly (76%) phosphatidylcholine (PC), about two thirds is dipalmitoyl phosphatidylcholine (DPPC), and the rest is unsaturated. 11% of the phospholipids are made up of phosphatidylglycerol (PG), 4% phosphatidylinositol, 3% phosphatidylethanolamine, 2% phosphatidylserine, 1.5% sphingomyelin and 0.2% lysophosphatidylcholine. Surfactant protein A (SP-A) represents 4% of surfactant and SP-B and SP-C and SP-D each make up less than 1%, according to current estimates.

SP-A and SP-D belong to the collectin subgroup of the C-type lectin superfamily. SP-A binds dipalmitoylphosphatidylcholine and SP-D binds phosphatidylinositol. SP-A also interacts with alveolar type II cells, implicating SP-A in surfactant phospholipid homeostasis. SP-B is required for the formation of tubular myelin from secreted lamellar body material.

Surfactant deficiency remains the most common and serious pulmonary affliction of premature infants. Surfactant deficiency is the major factor responsible for respiratory distress syndrome of the newborn (RDS) and for adult respiratory distress syndrome (ARDS). Since 1980, the exogenous administration of surfactant for the treatment of these syndromes is being studied.

A pathophysiological role for surfactant was first appreciated in premature infants with respiratory distress syndrome (RDS) and hyaline membrane disease. Use of exogenous surfactant and corticosteroid administration have made a major impact on improving survival and reducing morbidity in this disease with consequent alterations in the clinical and radiographic course.

Initial attempts at improving RDS with surfactant replacement during the 1960's (Chu et al., 1967) failed, largely because of a lack of knowledge about surfactant compositions and distributions. Liggins and colleagues (Liggins et al., 1972) where the first to utilise corticosteroids for the enhancement of fetal lung maturation, thereby reducing the risks and complications of RDS after birth. It is feasible that combining corticosteroids with thyroid-releasing hormone will enhance prenatal prophylaxis for RDS, and also inositol can be given as a substrate for surfactant production to infants in the early course of RDS.

A number of approaches for the design and the use of surfactant replacement for RDS have also been tried. The most straightforward approach is to replace with whole human

surfactant. Human pulmonary lung surfactant can only be harvested by lavage procedures, though, which may disrupt its pre-existing biophysical and biochemical micro-organisation. As seen in a study by Hallman and co-workers, (Hallman et al., 1983), this preparation was successful in clinical trials, but because of the difficulties in obtaining large quantities of human surfactant, it is not in commercial production.

These limitations make the production of synthetic pulmonary lung surfactant desirable. A second approach is therefore to learn the functions of the various surfactant constituents and then construct surfactants from substitutes that might be more easily obtained or less expensive than the isolation of the natural products.

Exosurf is a commercially available preparation containing DPPC, hexadecanol and tyloxapol. Hexadecanol and tyloxapol mimic, to some degree, the functions of surfactant proteins, PG and other lipids in natural surfactant. Several groups have added surfactant proteins to lipids, designing the proteins to mimic structure and function of native surfactant proteins.

Furthermore, there are new strategies that add surfactant proteins to lipid mixtures that include formulating proteins using *de novo* peptide synthesis or recombinant DNA techniques (Yao et al., 1990).

An ideal therapeutic surfactant should share many of the attributes of any ideal therapy. It should be stable, readily available, easy to make, inexpensive and have an easy route of administration, a half-life consonant with the disease process, and fully understood mechanisms of action, metabolism and catabolism. It should have maximum efficacy for the disease without toxicity, intolerance, immunogenecity or side effects. It should mimic the effects of the natural pulmonary lung surfactant, improve the gas exchange in the lungs, improve lung mechanics, improve functional residual capacity, resist inactivation, display optimal distribution characteristics, and have a known clearance mechanism. Its use should completely reverse the primary disease process and repair or allow the body to repair secondary damage from the primary disease.

Available therapeutic surfactants are of two types: those that are prepared from mammalian lungs and those made from synthetic compounds. Bovine and porcine surfactants contain SP-B and SP-C, associated with phospholipids, but SP-A and SP-D

are only present in the whole natural surfactant derived from amniotic fluid which, as described above, is not available commercially. The synthetic surfactants that are commercially available at present are Exosurf, Surfactant TA and a 7:3 mixture of DPPC and PG (ALEC).

The commercially available surfactants are all distributed as ready-mixed liquids, but Exosurf and Surfactant TA are supplied as a lyophilised powder that has to be reconstituted with saline. Surfactant TA is prepared by organic solvent extractions of bovine lung mince and is at present not available outside of Japan.

Surfactant therapy is at present an established part of routine clinical management of new-born infants with RDS. An initial dose of about 100 mg/kg is usually needed to compensate for the deficiency of alveolar surfactant in these babies, and repeated treatment is required in many cases. Recent experimental and clinical data indicate that large doses of exogenous surfactant may be beneficial also in conditions characterised by inactivation of surfactant, caused by, for example, aspiration of meconium, infection, or disturbed alveolar permeability with leakage of plasma proteins into air spaces.

The acute response to surfactant therapy depends on the quality of the exogenous material (modified natural surfactant is generally more effective than protein-free synthetic surfactants), timing of treatment in relation to the clinical course (treatment at an early state of the disease is better than later treatment and may reduce the subsequent need for mechanical ventilation) and mode of delivery (rapid instillation via a tracheal tube leads to a more uniform distribution and is more effective than slow airway infusion). Treatment with aerosolised surfactant improves lung function in animal models of surfactant deficiency, but is usually associated with large loss of the nebulized material in the delivery system. Furthermore, data from experiments on immature new-born lambs indicate that treatment response may depend on the mode of resuscitation at birth, and that manual ventilation with just a few large breaths may compromise the effect of subsequent surfactant therapy. The widespread clinical use of surfactant has reduced neonatal mortality and lowered costs for intensive care in developed countries.

The most efficient surfactants at present are prepared from mammalian lungs. The yield is very low and the therapy is therefore very expensive, therefore there is an urgent need to improve their efficiency and to standardize their application.

Summary of th invention

The present invention relates to a dynamic process of spreading that takes place during a defined span of time following the reconstitution of a lyophilised pulmonary lung surfactant (LS) in a physiological electrolyte solution. The lipid-protein bilayer structure is found to be organized towards the active establishment of an equilibrium composition, which is microscopically perceptible as the formation of a bifringent complex network. A LS composition for administration at a predetermined time point is provided and the means to determine said optimal time point for various LS extracts.

A more active spreading of said surfactant into the alveoles is obtained, improving the use of this composition as carrier for therapeutic components. The invention thus offers a composition and method for an improved treatment of respiratory distress syndrome (RDS) and other pulmonary diseases that are associated with deficiency of surfactant, as well as a more efficient means for the delivery of a number of therapeutic compounds.

Figure leg nds

Figure 1. A sample of 5% PLS and 95% Ringer solution viewed in the polarizing microscope 1 minute after mixing. The PLS particles under swelling accumulate at the surface towards air.

Figure 2. The sample as shown in Figure 1, shown 8 minutes after mixing. The birefringence increases at the tree-like surface which is formed towards air.

Figure 3. The birefringence of the surface network which has reached a steady state 19 minutes after mixing of the same sample as shown in Figure 1 and Figure 2. The polarizer and analyser of the microscope were perfecrly crossed, whereas the same deviation from 90° was employed in Figure 1 and Figure 2 in order to better see the surface of the droplet.

D tail d Discription

The present invention is based upon the surprising finding that an electrolyte containing solution, such as for example a Ringer solution, contrary to pure water, induces a highly dynamic swelling behaviour of a dispersion during the early stages of dispersing lyophilised LS in said solution. During this swelling period, the lipid-protein bilayer structure is found to be organised towards the active establishment of an equilibrium conformation. This process involves spreading at an interface and can be followed in the polarizing microscope as formation of birefringent networks, which is illustrated in figures 1 –3 (described in further detail beneath). Birefrigence is due to the occurrence of different refractive indexes in different directions of the sample, and it proves the occurrence of crystalline or liquid-crystalline order within the sample. Polar lipids like those in lung surfactant are known to form liquid-crystalline phases in water solutions. It is thus the optical character of these phases that can be followed by their birefringence.

The dynamic changes that can be observed are related to the presence of positive ions, such as sodium, potassium, calcium and magnesium ions. The physiological relevance of the effects of this dynamic behaviour has further been tested in a lung environment and in animal studies, showing significant clinical effects.

The unexpected physiological effects described above provide a new and improved means for the clinical use of lung surfactant extracts. According to the present invention, the lipid-protein surfactant should thus be administrated into the lungs together with a physiological electrolyte containing solution in a time-controlled fashion.

The present invention relates to the use of a molecular mixture comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract, or a semisynthetic or even a fully synthetic lung surfactant extract, said mixture being dispersed in a physiological electrolyte solution, for the preparation of a composition for administration at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

To determine the optimal time point for administering a composition in accordance to the present invention, samples containing LS dispersed in a physiological electrolyte solution are prepared and parts from that mixture ar regularly examined in a polarizing

microscope. At first, a homogeneous appearance will be obtained and the sample will be turbid with a viscosity like water. A view of the sample at this stage in the polarizing microscop will resemble figure 1. Small particles with a weak birefringence surrounded by the electrolyte solution can be seen accumulated at the outer boundary of the liquid phase towards water. The birefringency will then be observed to increase followed by a remarkable increase of contact surface area of the liquid phase towards air. Tubular formations grow out from the front of the liquid and they form branches, which successively become birefringent, comparable to those shown in figures 2 and 3. At approximately 30 minutes after adding the lyophilised LS to the physiological electrolyte solution, the surface zone will have developed into a static birefringent complex network.

The term 'birefringency' used herein means the separation of light, on passing through a crystal, into two unequally refracted, plane-polarized rays (of orthogonal polarizations). This effect occurs in crystals or liquid crystalls in which the velocity of light is not the same in all directions; that is, the refractive index is anisotropic.

As it is likely that the time for optimal physiological administration varies with the concentration and nature of the components of a a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation, even between batches of the same lyophilised LS, and also with the nature of electrolytes in the solution, it is necessary to determine the optimal time for each and every preparation by a standardised procedure.

The present invention thus relates to the use of a molecular mixture comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation, said mixture being dispersed in a physiological electrolyte solution, for the preparation of a composition for administration at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution, wherein said time point for administration has been determined microscopically as the half-time of the earliest time point at which the birefringence of the dispersion has reached a steady-state. By administration into the alveoli at this time maximal use is made of the dynamic spreading during surfactant molecular reconformation in water, induced by anion interaction.

The present invention further relates to a method for determining and standardising the period of time during which the aqueous swelling of lipids and proteins takes place in a molecular mixture of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation dispersed in a physiological electrolyte solution, said method comprising adding said mixture to said solution and observing the swelling kinetics in a polarised microscope, as described above and in example 1.

The time between mixing the dry powder in physiological electrolyte solution and the administration of said composition into the lungs for optimal effect is considered to be in the range of approximately 3–30 minutes. The present invention therefore relates to the administration of said LS composition at a time point that is at least 3 minutes and at most 30 minutes after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution, such as at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 minutes after adding said mixture, or such as at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 minutes after adding said mixture. As described above, the optimal time point for administering said composition will vary according to the quality of the dry powder and the choice of electrolyte solution that is used, and may even be longer than 30 minutes, such as between 30 and 45 minutes, or in certain cases even longer.

The term 'extract ' is herein used to describe LS prepared from lungs wherein cellular material has been removed by filtration and centrifugation before extraction, and wherein the organic solvent used for extraction successively has been replaced by water during freeze-drying to give a lyophilised powder.

The lyophilised lipids and proteins used in the present invention are preferably derived from porcine lung surfactant extract (PLS), but as the person skilled in the art will easily comprehend, they can as well be derived from any other mammalian origin, or even be synthetically produced. In one embodiment of the invention, the PLS is prepared from freshly slaughtered pigs. The pig lungs are minced and washed in saline solution and the mixture of proteins and lipids are then filtrated and centrifuged. Successively, the supernatant is ultracentrifuged and the pellet of crude surfactant extracted as described

(Bligh et al., 1959). The chloroform paste is evaporated and neutral lipids removed by acetone. The preparation is finally freeze-dried.
In one embodiment of the invention, said lyophilised lipids and proteins are thus obtained from a mammalian lung surfactant extract, but said lyophilised lipids and proteins can as well be obtained from alveolar cell cultures, or alternatively, be obtained chemically or synthetically.

Lung surfactant lipids and/or proteins can be obtained by culturing of lung cells and harvesting of the secreted lipids and/or proteins by methods well known to the person skilled in the art. Cell culture of lung surfactant is e.g. possible by use of the available *ATCC* cell line A549 (ATCC, 10801 University Boulevard, Manassas, VA 20110-2209, USA), which is derived from a human adenocarcinoma of the lung. One especially preferred embodiment of this invention therefore relates to the use of a molecular mixture comprising lyophilised lipids and proteins derived from a cell line.

The extract described above contains hydrophobic proteins and phospholipids. It further contains cholesterol, free fatty acids and fatty acid glycerides. In one embodiment of the invention, the extract thus comprises lyophilised surfactant proteins and lipids that are selected from a group consisting of phospholipids, DPPC, PG, fatty acids, hexadecanol, SP-A, SP-B, SP-C and SP-D. In another embodiment of the invention, the extract further comprises synthetic phospholipids and at least one of the hydrophobic proteins SP-B or SP-C. Said proteins can as well be recombinant proteins.

Both SP-B and SP-C present in the LS extract used in the present invention are basic proteins under physiological pH-values, like those from nerve myelin. The lipids are both anionic, which can form electrostatic complexes with the cationic protein, and zwitterionic (PC).

In the present invention, an aqueous electrolyte mixture of LS is prepared by adding an electrolyte solution, for example Ringer solution, to a predetermined amount of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation, into a glass test tube, which is gently shaken in order to facilitate mixing and interaction towards equilibrium. In a preferred embodiment of the invention, Ringer acetate from Pharmacia&Upjohn (Sweden) is used, which consists

of 130mmol Na^+, 4mmol K^+, 2mmol Ca^{2+}, 1mmol Mg^{2+}, 30mmol Ac^-, and 100mmol Cl^-. In another embodiment, said physiological electrolyt solution further also comprises SP-A.

The microscopic observations of the swelling processes of the present invention indicate that both the presence of the inorganic ions of the electrolyte solution and the presence of a solid/liquid interface is needed for the remarkable formation of such surface network textures as seen in figures 2 and 3. This process represents a dynamically active state of a LS. Extraction in an organic solvent and evaporation, as in the LS extract or preparation used in the present invention, means that polar regions are turned inside and hidden by hydrocarbon regions. The PG/SP-B and PG/SP-C ionic complexes must therefore change their conformations drastically in order to form bilayers, when exposed to water. This takes some time. When ions from saline or Ringer solution are present, they may contribute to the dissociation of these complexes.

Any reorganisation within the bilayer is to be expected to induce increased dynamics. This is probably the reason behind the elaborate birefringent network formation following the dispersion of lyophilised LS into Ringer solution. There is always a driving force at exposed interfaces to reduce the surface free energy, and the reorganisation process should favour the formation of low-energy interfaces towards glass and air. This mechanism also explains why the network is not observed in LS samples swollen in distilled water.

In the present invention, said physiological electrolyte solution is therefore selected from the group consisting of saline (physiological sodium chloride) solution and a Ringer solution. In a preferred embodiment said physiological electrolyte solution is a Ringer solution.

The calcium concentration should be physiologically acceptable, and therefore in one embodiment of the invention, the composition comprises a calcium concentration close to the extracellular concentration in the lung alveoli. In another preferred embodiment of the invention, the coposition comprises proportions (weight/weight, w/w) of said lyophilised proteins and lipids to said electrolyte solution that are about 1:99, 2:98, 3:97, 4:96, 5:95, 6:94, 7:93, 8:92, 9:91,10:90, 11:89, or 12:88, the most preferred embodiment comprising proportions (weight/weight, w/w) of said lyophilised proteins and lipids to said electrolyte containing solvent that are about 6:94.

It is assumable that the results described are also valid in other lung surfactant extracts, for example from bovine lungs.

The most important feature of the present invention is the unexpected finding of an optimal time after mixing LS extract and/or LS preparation and a solution with regard to the physiologic effect after administration. The present invention includes the observation that this optimal time can vary from one batch of LS extract and/or LS preparation to another. Predetermination of the time optimum means that the time optimum for each batch of applied LS extract and/or LS preparation and for each type of electrolyte used is determined by a standardised procedure as described above, meaning each LS composition is standardised for optimal performance. This time optimum will also vary with concentration of LS extract and/or LS preparation in the aqueous phase and with the type of electrolyte used. The concentration of LS extract in the final composition is generally within the range of 1-100mg/ml LS, such as at least 1mg, 2mg, 3mg, 4mg, 5mg, 6mg, 7mg, 8mg, 9mg, 10mg, 15mg, 20mg, 25mg, 30mg, 40mg, 50mg, 60mg, 70mg, 80mg, 90mg, and/or at least 100mg. A concentration of 5 mg LS extract /ml is generally preferred. The time optimum can also vary somewhat between saline and Ringer solution. A good agreement between the biologically determined time optimum and the half-value of the time required to reach a steady-state birefringence front of the formulation as seen in the polarising microscope was found to exist in rat studies, and forms the basis of the present invention. It is known since earlier, by the person skilled in the art, that the results from rats can be applied in human therapy. It is therefore concluded that the data derived from the rat studies are relevant also in humans. No such time effects at administration have been observed earlier.

The present invention also offers the possible use of the swelling behaviour of a composition comprising a molecular mixture of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation dispersed in a physiological electrolyte solution as observed microscopically for determining the quality of an LS extract and/or LS preparation. A method to standardize quality is valuable as the dose required in therapy is related to the performance quality as seen in the dynamic spreading behaviour.

The optimal concentration for lung administration of the surfactant in saline or Ringer solution is considered to be in the range between about 4–10 %(w/v). For use in the

present invention, a concentration of 5 % (w/v) is considered as ideal with regard to the administration through a tracheal tube and the limitation of administering reasonable volumes. A preferred embodiment of the invention thus relates to a composition that contains lyophilised lipids and proteins, such as at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9% or at least 10%.The most preferred embodiment relates to a composition that contains at least 5% lyophilised lipids and proteins.

The present invention relates to the use of the surprising finding that there is a dynamic process of spreading during a defined span of time following the reconstitution of a lyophilised lung surfactant in a physiological electrolyte solution. Hereby, a more active spreading of said surfactant into the alveoles is obtained and the possibility is given to use this process for an improved treatment of lack of lung surfactant in a subject in need thereof.

One embodiment of the present invention relates to the use of an optimal process of spreading of a lung surfactant composition comprising a molecular mixture comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation dispersed in a physiological electrolyte solution, for improved administration of said composition into the alveoli of a subject, characterised by administration of said lung surfactant composition at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution. Said subject can be a human.

The physiological effects used in the present invention imply that at a clinical use of a surfactant extract and/or LS preparation according to the present invention, the lipid-protein surfactant should be administrated into the lungs together with a physiological electrolyte solution in a time-controlled fashion. Consequently, a method of treating a person in need thereof will comprise administering a molecular mixture of lipids and proteins from pulmonary lung surfactant extract or a fully synthetic lung surfactant preparation, reconstituted in a physiological electrolyte solution, into the alveoli of said person during a predetermined span of time during which said molecular mixture is displaying an active dynamic spreading.

The invention further relates to a method of treating or preventing a pulmonary lung disease in a subject, comprising dispersing a composition comprising a molecular mixture

comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant extract dispersed in a physiological electrolyte solution, and administering the composition into the alveoli of a subject in need thereof at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

Such a method as described above can be used for the treatment or prevention of respiratory distress syndrome (RDS), adult respiratory stress syndrome (ARDS), congenital diaphragmatic hernia, infants treated with Extracorporeal Membrane Oxygenation (ECMO), and/or meconium aspiration pneumonia.

In an especially preferred embodiment of the present invention, administration is performed via a tube into the lungs.

A composition comprised in the present invention can furthermore be of use as a carrier for other therapeutic components into areas that are hard to access and thus provide an improved transport of compounds over the water-air surface of lungs.

This kind of formulation and activation can also be used as a pulmonary drug delivery system for controlled release of therapeutically active molecules. Surfactants can serve as carriers or as vehicles for delivery of additional therapeutic agents such as brochodilators, anti inflammatory agents, histamine-receptor antagonists, inhalation steroids, DNA-ases, immunotherapy, vasodilators, antibiotics, growth factors, drugs enhancing epithelial integrity, factors accelerating lung maturation, anti-neoplastic drugs and/or gene-therapy. These potential uses of surfactant for pulmonary drug delivery would be applicable in particular in the following diseases: chronic obstructive lung disease, asthma, bronchopulmonary dysplasia, lung infections, persistent pulmonary hypertension, lung infections, lung hypoplasia, bronchopulmonary dysplasia (Vit A), respiratory distress syndrome, cancer, cystic fibrosis, alveolar proteinosis, and/or congenital SP-B deficiency.

Alternatively, the drug delivery system provided by the present invention can of course be as applicable for delivering drugs into a subject in need thereof, even if said subject does not suffer from a lung-related disease or a disease related to lung sufficiency. Such disease could for illustrative purposes only and not limitted to, for example be either cancer and/or diabetes.

Another possible field of use for the present invention is the treatment of patients after surgery, wherein the composition is applied in order to prevent or avoid adheranc formation between tissues in mutual contacts.

The field of pulmonary drug delivery is very active at present. The main delivery route is the oral delivery route, where many complications have been reported which do not exist in pulmonary delivery, such as degradation of the drugs by the low pH or any of the enzymes in the gastrointestinal tract. The physiological nature of the surfactant makes it ideal as a vehicle in delivery into the lungs of almost any drug used systemically.

Additionally, therapeutic agents based on the present invention comprise a pharmaceutical substance encapsulated in surfactant liposomes.

For easy administration in clinical use, the present invention also encompasses a three component kit for time controlled administration of a pulmonary lung surfactant composition, wherein a physiological electrolyte solution is achieved in the administered composition, containing

a) a lyophilised mixture of a pulmonary lung surfactant extract or a semisynthetical or fully synthetic lung surfactant preparation,

b) a salt, and

c) a written instruction containing information about the period of time during which the aqueous swelling of lipids and proteins takes place and how to use said kit. As described above, said lyophilised mixture of a pulmonary lung extract or preparation can contain varying mixtures of phospholipids and the proteins SP-B and/or SP-C. In a preferred embodiment of the invention, the administered LS is dispersed in Ringer solution.

Examples

Example 1

In vitro sample calibration and standardisation:

Aqueous samples of PLS were prepared by adding varying proportions of water, saline solution or Ringer solution in glass test tubes, which were gently shaken in order to facilitate mixing and interaction towards equilibrium, alternatively, the solution was sucked up and ejected by a syringe five times. Ringer-acetate from Pharmacia & Upjohn was used (Na^+ 130 mmol, K^+ 4 mmol, Ca^{2+} 2mmol, Mg^{2+} 1 mmol, Ac^- 30 mmol, Cl^- 100 mmol). Aliquots of equilibrated or freshly prepared samples were transferred to microscope slides for examination either during swelling of the dry PLS powder or after equilibrium had been reached. Observations in the microscope were performed at 25°C and sometimes at 42°C. A Leitz polarizing microscope was used with a Sony CD camera and colour printer.

Directly after mixing of the sample as defined above, a droplet is examined in the polarizing microscope as described below. A droplet was deposited on a slide and a coverslip was put down on the droplet very gently, in order to avoid air bubble incorporation. The appearance in the polarizing microscope of the interface between the droplet and air after 1 minute is shown in Figure 1. The PLS particles that are under swelling, accumulate at the surface towards air and show weak birefringence. The growth of tree-like textures at the interface starts successively, and the appearance at 8 minutes after mixing is shown in Figure 2. After 19 minutes from the timepoint of the mixing, a steady state in the dynamic surface reorganization was reached, and the appearance of this final network is shown in Figure 3.

The same experiments were performed with water replacing the Ringer solution. When water was added to the PLS, no such network formation could be observed.

Example 2

Animal experiments

White laboratory rats were anaesthetised using isoflurane. A tracheostomy was performed. Six rats at a time were connected to a Siemens-Elema Servoventilator 900 to give identical breathing cycles. Anaesthesia and muscle-relaxation were maintained with penthobarbithal and celocurine. The lungs were lavaged with physiological saline at 40°C to remove the endogenous surfactant. The lavage-procedure was repeated until the rats had a PO_2 below 80mm Hg. The surfactant solutions in a concentration of 50 mg/ml were given in a dose of 30mg/kg matched to the bodyweight of each animal (average dose 0.2 ml) followed by two air boluses of 2 ml each. Arterial bloodgases were taken before the lavaging procedure, after lavage, at 5, 30, 60, 90 and at 120 minutes after the surfactant instillation. The recorded mean arterial PO_2 values for each group is presented in table 1. The effect of the solutions administered 10 minutes after preparation are significantly superior to those administered at 40 minutes after preparation as represented by the higher PO_2 values.

When just water was used in the formulation of PLS, the physiological effects after administration were much weaker than any of the ion containing solutions.

(n= 4x6 rats were used in these experiments, control rats die)

Table 1.

Arterial PO2 saturation

	Ringer 10	Ringer 40	NaCl 10	NaCl 40
Before lav.	602	559.125	591.875	583.25
After lav.	58.25	62.75	51.875	59.25
5 min	383.125	381.142857	420	391
30 min	376.625	415.125	416.875	391.625
60 min	375.75	323.625	425.625	341.5
90 min	342.25	292	395.125	333.75
120 min	326.125	292.5	362.25	307

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Claims

1. Use of a molecular mixture comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation, said mixture being dispersed in a physiological electrolyte solution, for the preparation of a composition for administration at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

2. Use according to claim 1, wherein said time point for administration has been determined microscopically as the half-value of earliest time point at which the birefringence of the dispersion has reached a steady state.

3. Use according to claim 1 or 2, wherein said time point is at least 3 minutes and at most 30 minutes after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

4. Use according to any of claims 1-3, wherein said lyophilised lipids and/or proteins are obtained from a mammalian pulmonary lung surfactant extract.

5. Use according to any of claims 1-4, wherein said lyophilised lipids and proteins are obtained from alveolar cell cultures.

6. Use according to any of claims 1-3, wherein said lyophilised lipids and proteins are obtained synthetically.

7. Use according to any of claims 1-6, wherein said lyophilised lipids and proteins comprise surfactant proteins.

8. Use according to any of claims 1-7, wherein said lyophilised lipids and proteins comprise phospholipids.

9. Use according to any of claims 1-8, wherein said lyophilised lipids and proteins comprise DPPC.

10. Use according to any of claims 1-9, wherein said lyophilised lipids and proteins comprise components selected from a group consisting of DPPC, PG, fatty acids, hexadecanol, SP-A, SP-B, SP-C and/or SP-D.

11. Use according to any of claims 1-10, wherein said lyophilised lipids and proteins comprise synthetic phospholipids and protein SP-B or SP-C, or both proteins.

12. Use according to any of claims 1-11, wherein at least one of said proteins is a recombinant protein.

13. Use according to any of claims 1-12, wherein said physiological electrolyte solution is selected from the group consisting of saline(physiological sodium chloride) solution and Ringer solution.

14. Use according to claim 13, wherein said physiological electrolyte solution is Ringer solution.

15. Use according to claim 13 or claim 14, wherein said physiological electrolyte solution further comprises SP-A.

16. Use according to any of claims 1-15, wherein said composition contains 4-10%(w/v) lyophilised lipids and proteins.

17. Use according to claim 16, wherein said composition contains 5%(w/v) lyophilised lipids and proteins.

18. Use according to any of claims 1-17, for the treatment of lack of lung surfactant.

19. Use according to any of claims 1-18, for lowering the surface tension of the air-water interface of a mammalian lung.

20. Use of an optimal process of spreading of a lung surfactant composition comprising a molecular mixture comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation dispersed in a physiological electrolyte solution, for improved administration of said

composition into the alveoli of a subject, characterised by administration of said lung surfactant composition at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

21. Use according to claim 20, wherein said time point for administration has been determined microscopically as the half-value of the earliest time point at which the birefringence of the dispersion has reached a steady state.

22. Use according to claim 20 or 21, wherein said time point is at least 3 minutes and at most 30 minutes after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

23. Use according to any of claims 20-22, wherein the subject is a human.

24. Use according to any of claims 20-23, for the treatment or prevention of respiratory distress syndrome (RDS), adult respiratory stress syndrome (ARDS), congenital diaphragmatic hernia, infants treated with ECMO, and/or meconium aspiration pneumonia.

25. Use according to claim 24, for the treatment or prevention of respiratory distress syndrome (RDS).

26. A method of treating or preventing a pulmonary lung disease in a subject, comprising dispersing a composition comprising a molecular mixture comprising lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation in a physiological electrolyte solution, and administering the composition into the alveoli of a subject in need thereof at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

27. A method according to claim 26, wherein said time point for administration has been determined microscopically as the half-value of the earliest time point at which the birefringence of the dispersion has reached a steady state.

28. Use according to claim 26 or 27, wherein said time point is at least 3 minutes and at most 30 minutes after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

29. Use according to any of claims 1-23 for pulmonary drug delivery.

30. Use according to claim 29, which comprises a pharmaceutical substance encapsulated in surfactant liposomes.

31. Use according to any of claims 1-23 for pulmonary delivery of therapeutic proteins.

32. Use according to any of claims 29-31, for treatment of chronic obstructive lung disease, asthma, bronchopulmonary dysplasia, lung infections, persistent pulmonary hypertension, lung infections, lung hypoplasia, bronchopulmonary dysplasia (Vit A), respiratory distress syndrome, cancer, cystic fibrosis, alveolar proteinosis, and/or congenital SP-B deficiency.

33. Use according to any of claims 29-31, for preventing adherence formation between tissues in mutual contact.

34. A method of producing a composition comprising a molecular mixture of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation, characterized by
a) dispersing said lyophilised lipids and proteins in a physiological electrolyte solution,
b) administering the composition at a predetermined time point after adding said mixture comprising lyophilised lipids and proteins to a physiological electrolyte solution.

35. A method according to claim 34, wherein said time point for administration has been determined microscopically as the half-value of the earliest time point at which the birefringence of the dispersion has reached a steady state.

36. Use according to claim 34 or 35, wherein said time point is at least 3 minutes and at most 30 minutes after adding said mixture comprising lyophilised lipids and proteins to said physiological electrolyte solution.

37. A method for activating and administering a molecular mixture of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation, characterized by

a) dispersing said lipids and proteins in a physiological electrolyte solution, and

b) administering said dispersed lipids and proteins at a predetermined optimal time point, characterised as the half-value of the time at which the aqueous swelling of said lipids and proteins has reached a steady state.

38. A method according to claim 37, wherein the defined period of time is at least 3 minutes and at most 30 minutes after reconstitution of said lyophilised lipids and proteins.

39. A method according to claim 37 or 38, said method being characterized by

a) evaporating the water from a liquid formulation of said lipids and proteins,

b) resolving said lyophilised lipids and proteins in a physiological electrolyte solution,

c) administering said resolved lipids and proteins during a defined period of time, wherein the actual swelling of said lipids and proteins takes place.

40. A method for determining and standardising the period of time during which the aqueous swelling of lipids and proteins takes place in a molecular mixture of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation dispersed in a physiological electrolyte solution, said method comprising adding said mixture to said solution and observing the swelling kinetics in a polarizing microscope.

41. A three component kit, for time controlled administration of a pulmonary lung surfactant composition, containing

a) a lyophilised mixture of a pulmonary lung surfactant extract,

b) a salt, and

c) a written instruction how to use said kit, containing information about the period of time during which the aqueous swelling of lipids and proteins takes place after the addition of a specified amount of sterile distilled water,

wherein a physiological electrolyte solution is achieved in the administered composition.

42. A three component kit, for time controlled administration of a pulmonary lung surfactant composition, containing

a) a lyophilised mixture of phospholipids and the proteins SP-A, SP-B and/or SP-C,
b) a salt, and
c) a written instruction how to use said kit, containing information about the period of time during which the aqueous swelling of lipids and proteins takes place,
wherein a physiological electrolyte solution is achieved in the administered composition.

43. A three component kit, for time controlled administration of a pulmonary lung surfactant composition, containing
a) a lyophilised mixture of phospholipids and the proteins SP-B and/or SP-C,
b) a salt, and
c) a written instruction how to use said kit, containing information about the period of time during which the aqueous swelling of lipids and proteins takes place,
wherein a physiological electrolyte solution is acchieved in the administered composition.

46. Use of the swelling behaviour by quantification of birefringence formation and steady state time of a composition comprising a molecular mixture of lyophilised lipids and proteins from a pulmonary lung surfactant extract or a semisynthetic or fully synthetic lung surfactant preparation dispersed in a physiological electrolyte solution as observed microscopically for defining quality parameters of a composition.

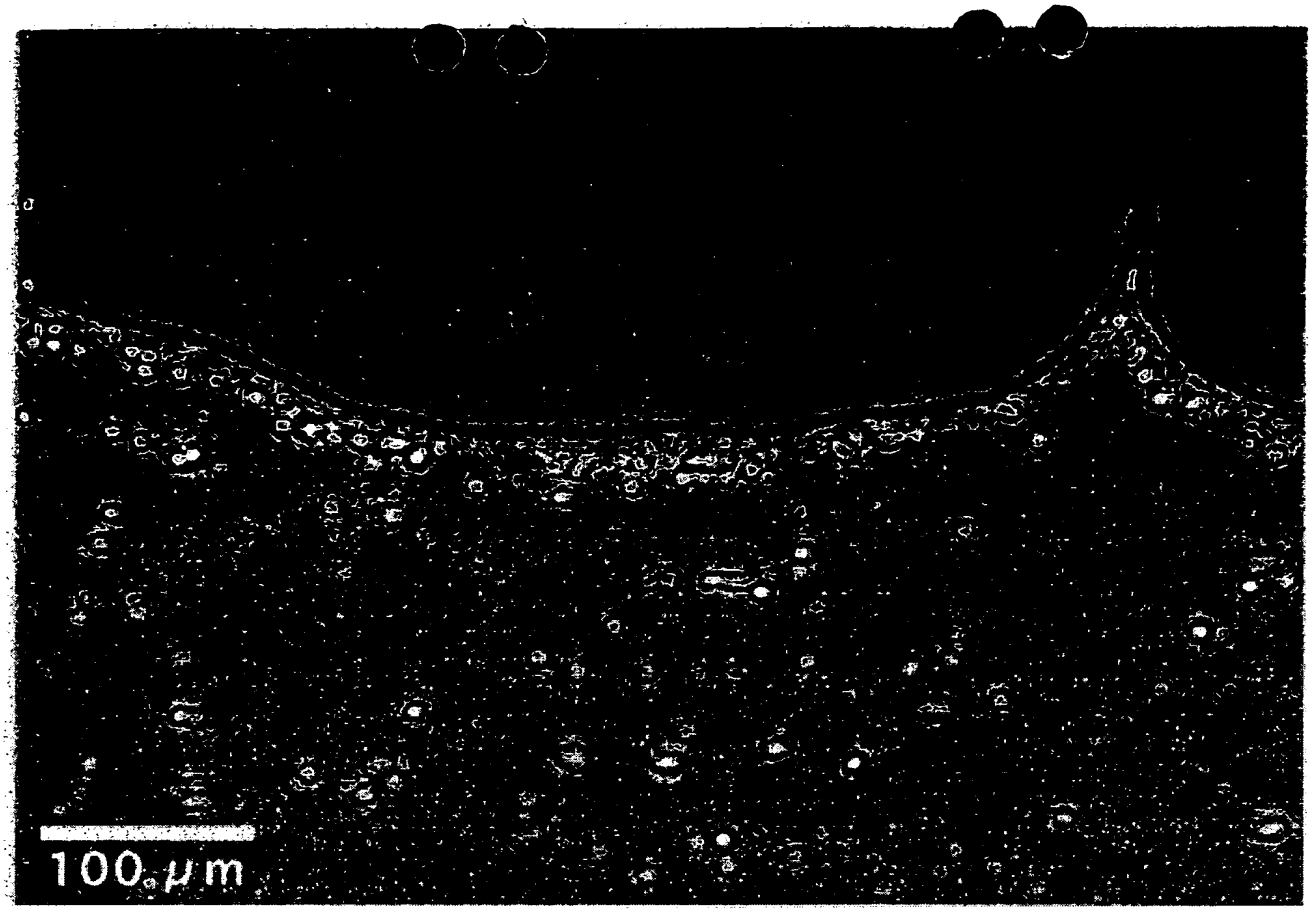

FIG 1

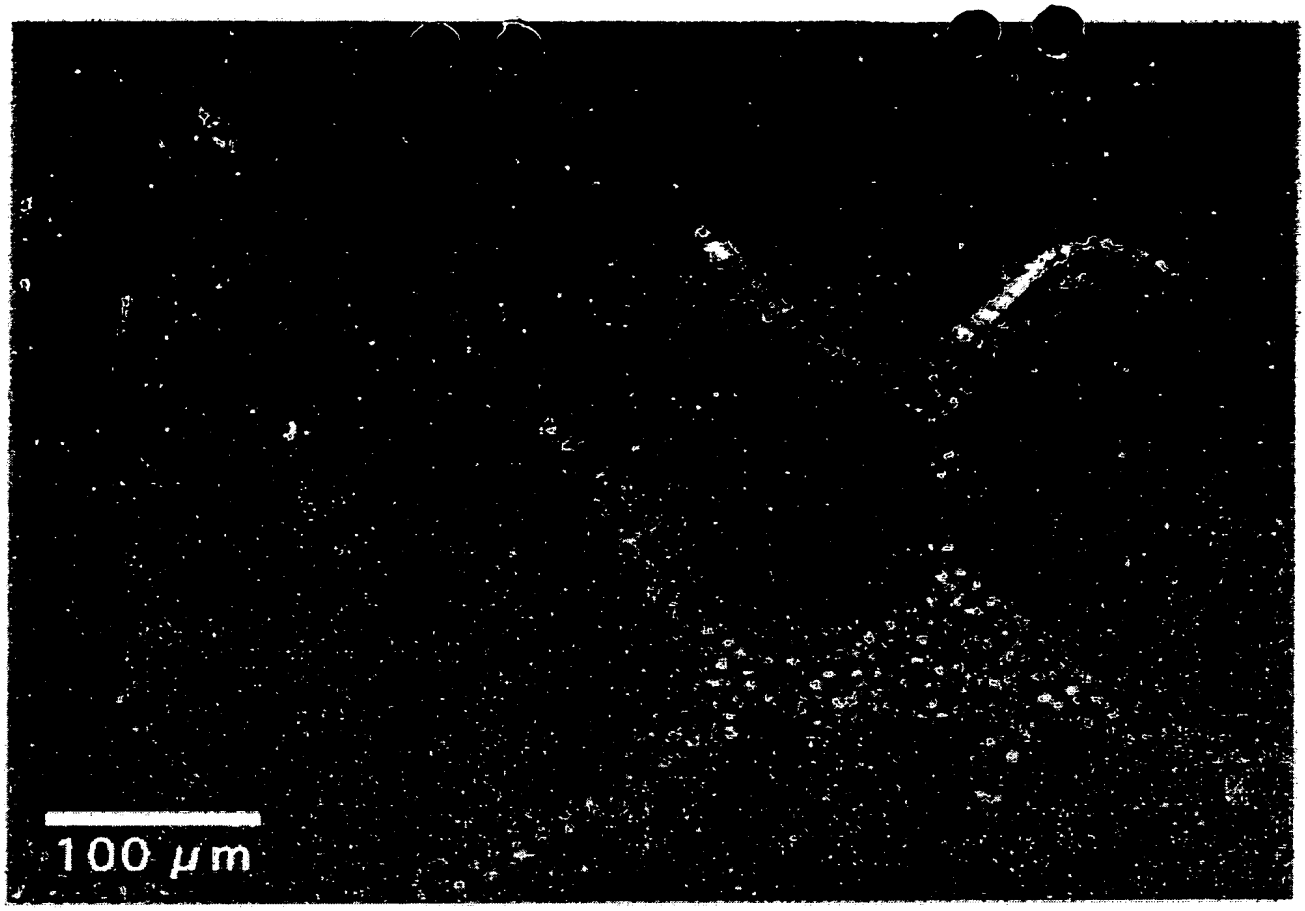

FIG 2

FIG 3